RESEARCH

24(101), January - February, 2020

ISSN
2321–7359
EISSN
2321–7367

Medical Science

CDK1 and PLK1 are key regulator proteins in human Papilloma virus Type 16-Positive Cervical Cancer: A Network-Based Study

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Article History
Received: 04 September 2019
Reviewed: 07/September/2019 *to* 25/October/2019
Accepted: 27 October 2019
Prepared: 29 October 2019
Published: January - February 2020

Citation
Seyedeh Zahra Mousavi, Vahdat Poortahmasebi, Talat Mokhtari-Azad, Shohreh Shahmahmoodi, Somayeh Jalilvand, Ezatollah Rafiei Alavi. CDK1 and PLK1 are key regulator proteins in human Papilloma virus Type 16-Positive Cervical Cancer: A Network-Based Study. *Medical Science*, 2020, 24(101), 201-214

ABSTRACT

Human papillomavirus (HPV) is the causative agent of cervical cancer. The purpose of present study was to provide a comprehensive protein-protein interaction (PPI) approach to identify the representative sub-networks for this cancer. Comprehensive gene expression profiling of cervical samples from different stages were collected from Gene Expression Omnibus (GEO-dataset: GSE67522). Among the three clinical stages, we generated two PPIs including, pre-cancerous network (normal-non-malignant) and cancerous network (non-malignant-CC or normal-CC). Subsequently, further bioinformatics analyses were performed. GO analysis revealed that the majority of differentially expressed genes in reconstructing cancerous networks was obviously involved in cell cycle processes. Serine/threonine kinases Polo-like kinase 1 (PLK1), cyclin-dependent kinase 1 (CDK1), and CDK2 were the most important hub genes in the protein-protein interaction network. These proteins play critical roles in the dysregulation of the cell cycle in cervical cancer development. Also, other cell cycle associated genes including AURKA, BRCA1, and CDC20 were all found highly critical genes in HPV16-infected cervical cancer. CDK1, CDK2 and PLK1 play essential roles in mediating integrative genetic networks involved in the development of cervical cancer. These hub genes might help improve pathogenesis of cervical cancer and may be used for the diagnosis and treatment of this cancer.

Keywords: Human papilloma virus, protein-protein interaction, cervical cancer, gene ontology, serine/threonine kinases Polo-like kinase 1, cyclin-dependent kinase 1

1. INTRODUCTION

Cervical cancer is the fourth most frequent cancer among women worldwide (about 500,000 new cases annually) and a major cause of cancer deaths in developing countries(Bray et al., 2013). Human papilloma virus (HPV), the major etiologic contributor to the development of cervical cancer, is found in almost all cases of invasive cervical cancer (ICC) (Pisani et al., 1999). Approximately, 40 HPV types can infect the genital tract. However, only a subset of these types is found regularly in cervical cancers, which designated as high-risk. Although more than 20 HPV types were found in the tumor, four types (HPVI6, HPV18, HPV31, and HPV45), account for close to 80% of ICC cases. HPV16 alone is responsible for more than 50% of the cervical cancer cases worldwide (zur Hausen, 1996; Doorbar et al., 2015).

HPV-16 DNA integration into the host genome is characteristically associated with disruption of the viral E1 and E2 genes, which permit higher transcription of E6 and E7 oncoproteins. These are HPV transforming proteins, which are able to form a complex with pRB and p53 and other cellular proteins. Consequently, the activity of these oncoproteins results in the progression of the cell cycle from the G1 phase to the S phase, which is a crucial point in the cell cycle process. Several HPV genes play essential roles in cell cycle progression. However, the spectrum of temporal pathway deregulation has not been investigated using a systematic framework. Gene expression profiling based on microarrays data has been performed broadly in facilitating cancer diagnosis such as leukemia (Golub et al., 1999), diffuse large B-cell lymphoma (DLBCL) (Alizadeh et al., 2000), and breast cancer (Sorlie et al., 2001).

Network-based approaches will provide a better understanding of the cellular networks, which may lead to design better targets for drug development (Barabasi et al., 2011). One of the most useful approaches in providing a global overview of cancer pathways and the representation of multiple interactions within a cell has been reconstruction of integrative protein-protein interaction (PPI) networks using several algorithms or interaction databases (Blais & Dynlacht, 2005; Emmert-Streib et al., 2014). The availability of genome-wide gene expression such as microarray data has helped to develop several state of the art PPI reconstruction methods (Margolin et al., 2006; Butte AJ, Kohane, 2000). In a previous study, a network-based attitude to the role of hepatitis C virus in the molecular pathways of hepato cellular carcinoma was applied. The results demonstrated that some critical genes and microRNAs were involved in the development of liver cancer patients (Poortahmasebi et al., 2016). PPI have not yet been applied widely to the investigation of cervical cancer. Therefore, the aim of the current investigation was to demonstrate the important disease related genes for HPV16 cervical cancer, cellular pathways, and protein-protein interactions by integrative microarray profiling data and network analysis at a systematic level.

2. MATERIALS AND METHODS

Microarray Data Preparation

Microarray gene expression data were acquired from the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) dataset (GSE67522, platform: GPL10558). This dataset consisted of the microarray profile of 12 histologically normal cervical tissues

(HPV negative), 10 HPV 16 positive non-malignant lesions, and 18 HPV16 positive ICC cases. GEO2R was utilized to identify the differentially expressed genes (DEGs) in these samples. After samples have been assigned to specific groups, we selected the top 250 genes as seed genes to run the test with default parameters. GEO2R employs the GEO query and limma R packages from the Bioconductor project to compare the gene expression on original submitter-supplied processed data. The GEO2R does normalize the data with log2 transformation along with false discovery rate (FDR) and provides the annotation information (P-value of <0.05) (Barrett et al., 2013).

Another independent microarray gene expression dataset (GSE9750, platform: GPL96) was used to further validate our results. This data set consisted of compatible HPV negative control (n= 24) and HPV16 positive CC samples (n= 19), which we analyzed to confirm our Normal-CC network. We excluded the samples from CC patients with non-type 16 HPV. The DEGs clustering was performed using Heat map Hierarchical-clustering analysis based on the average linkage method. Tree view analysis of DEGs in normal versus CC was carried out and displayed by Treeview 1.1 software. The study protocol was in accordance with the Helsinki Declaration and confirmed by the Ethics Committee of Tehran University of Medical Sciences (Approval Code: IR.TUMS.REC.1396.293).

Reconstruction of PPI Networks

The PPI network was created using the BisoGenet plugin of Cytoscape software (Cytoscape version 3.7.1, USA) and then the interactions between genes were retrieved and displayed as an interaction network. BisoGenet plugin permitted seeking from well-recognized interaction databases including, Biological General Repository for Interaction Datasets (BioGRID), Database of Interacting Proteins (DIP), Molecular INTeraction database (MINT), Human Protein Reference Database (HPRD), Biomolecular Interaction Network Database (BIND) and IntAct (Xenarios et al., 2002; Stark et al., 2011; Bader et al., 2003; Prasad et al., 2009; Zanzoni et al., 2002; Kerrien et al., 2011).

Detection of Functional Modules and Enrichment of DEGs

'Module' is defined as a series of interacting molecules that are closely related to one another. Modules in a PPI, for instance, are indicative of vital functional protein complexes. MCODE (Bader & Hogue, 2003), one of the most generally used Cytoscape plugins, has been designed to execute network module identification specifically in biology. MCODE groups a given network based on the principles that depend on local neighborhood density and outward traversal from a locally dense seed protein to separate the highly interconnected regions, and subsequently displays extracted modules and associated information.

The annotated function of genes within a network can give useful information about the function of a network. Therefore, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to demonstrate the role of hub genes in biological processes in tissues of samples (Huang et al., 2009; Huang et al., 2009) (P value< 0.05 considered significant). The Biological Networks Gene Ontology (BiNGO) plugin of Cytoscape was also used to find enriched functions in a given gene list. BiNGO (Maere et al., 2005) assess statistically over represented functions (Gene Ontology [GO] terms) in the biological network, or any other set of genes. In addition, DAVID tools were applied for the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses with a P-value set to <0.05. The KEGG is an authoritative database containing a variety of biochemical pathways (Kanehisa & Goto, 2000).

We acquired the intersection between the DEGs and gene ontology terms. Then, we obtain both the important GOs and the genes related to the significant GO terms. The DEG-GO network was constructed using Cytoscape software.

Network Topological Analysis

To predict the critical regulators in the cervical cancer based on hub gene variations, topological network analysis were calculated for three reconstructed PPIs (normal-HPV16, HPV16-CC, and normal-CC) with the Network Analyzer plugin. This plugin explores important nodes/hubs and fragile motifs in an interactome network by several topological algorithms. In the present study, three reliable topological factors including, Degree centrality, Closeness centrality, and Betweenness centrality were considered a criterion for identifying hub genes owing to their precise output. Centrality measures quantify such facts from different points of view (Ghasemi et al., 2014). Degree is defined as the number of links incident upon a node (gene), and measures the involvement of the node in the PPI network. Usually, genes which have higher degree within biological networks play a biologically important role in the regulation of several cellular functions. Closeness centrality was defined as the inverse sum of the shortest distances to all other nodes from a focal node. The closeness centrality of each gene is a number ranged between 0 and 1. The Betweenness centrality measures the degree to which a gene lies on the shortest path between two other nodes, and are able to funnel the flow in the

network. A node with high betweenness centrality has a large effect on the transfer of items through the network, under the presumption that item transfer follows the shortest paths.

Interactions between HPV Proteins and Network Proteins

A human-virus PPI network was reconstructed using Hu-Vir database (http://cdfd.org.in/labpages/computational_biology_data sets.html) to characterize the HPV target genes in the obtained differentially expressed gene lists. Hu-Vir database were retrieved from databases available on the pathogen interaction gateway (PIG) [http://pathogenportal.net/pig/] and viral protein interaction database (VirusMINT) [http://mint.bio.uniroma2.it/virusmint/Welcome.do], as well as literature-based information.

3. RESULTS

Global Properties Protein-Protein Interaction Networks

12 patients with CC, 10 non-malignant patients, and 18 healthy controls were enrolled in this study, and 43 subjects of another dataset were assigned to a validation group (GEO dataset: GSE9750). A PPI was created for each deferentially expressed gene list resulted from analysis of pre-cancerous (normal-nonmalignant) and cancerous (nonmalignant-CC and normal-CC) microarray gene expression profiles. Previously, hierarchical-clustering analysis based on the normalized expression of DEGs indicated that all of the patients with CC were clustered together, which indicated that these DEGs can be used to demarcate patients with CC from those with histopathologically normal subjects (HPV negative and HPV16 positive) (Sharma et al., 2015). The number of nodes-edges in the PPI networks of normal-nonmalignant tissue, non-malignant-CC and normal-CC were 154-27, 221-207, and 209-249, respectively. More information of each PPI networks is represented in Table 1. We found little overlap between pre-cancerous and cancerous protein-protein interaction networks (less than 2%).

Table 1 General properties of protein-protein interactions (PPIs)

Parameter	Definition	Normal-non malignant	Nonmalignant-CC	Normal-CC
Number of nodes	Component of a network (e.g., a gene or a protein)	154	222	209
Number of edges	Number of interaction between two nodes	27	231	249
Network diameter	The highest distance between two nodes	7	7	8
Network radius	The smallest distance between two nodes	1	1	4
Network centralization	How does the network topology resemble a star structure? (A value between 0 and 1)	0.019	0.098	0.113
Shortest paths	Minimum numbers of edges form a path. Shortest paths defined as distance	16 (0%)	6340 (12%)	8190 (18%)
Characteristics path length	Distance between two connected nodes	1.25	3.159	3.277
Network density	How densely is the network populated with edges? (A value between 0 and 1)	0.001	0.007	0.008
Clustering coefficient	A measure of the degree to which nodes in a graph tend to cluster together	0.015	0.071	0.092
Network heterogeneity	Tendency of a network to contain hub nodes	4.746	2.093	1.998
Number of connected components	Number of sub-networks that constitute a network	149	134	119
Average number of neighbors	The average connectivity of a node	0.078	1.486	1.761

CC: Cervical cancer

All sub-networks of the PPIs were extracted by the Biso Genet plugin and the main sub-network was determined subsequently from each PPI network. Numbers of sub-networks that constitute normal-nonmalignant, nonmalignant-CC and normal-CC networks were determined as 149, 134 and 119, respectively. Pairwise connected nodes (genes) erroneously increased the number of sub-networks. Our analysis also demonstrated that some of the genes were common between different networks (Figure 1).

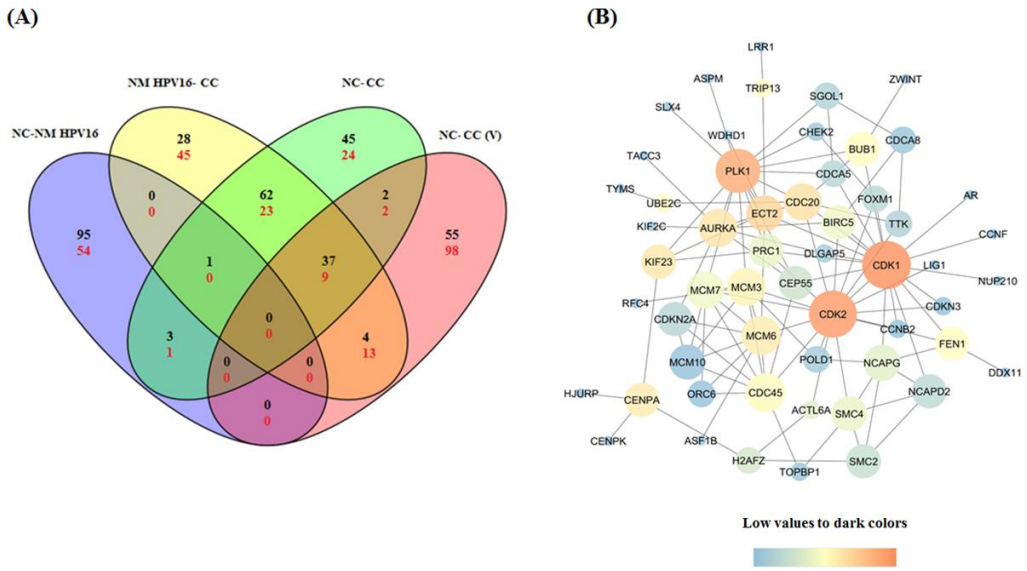


Figure 1 (A) Venn diagram of the differentially expressed genes (DEGs). Numbers of upregulated genes are shown in black and downregulated in red, (B) Network obtained from common DEGs of NM HPV 16-CC and NC-CC.

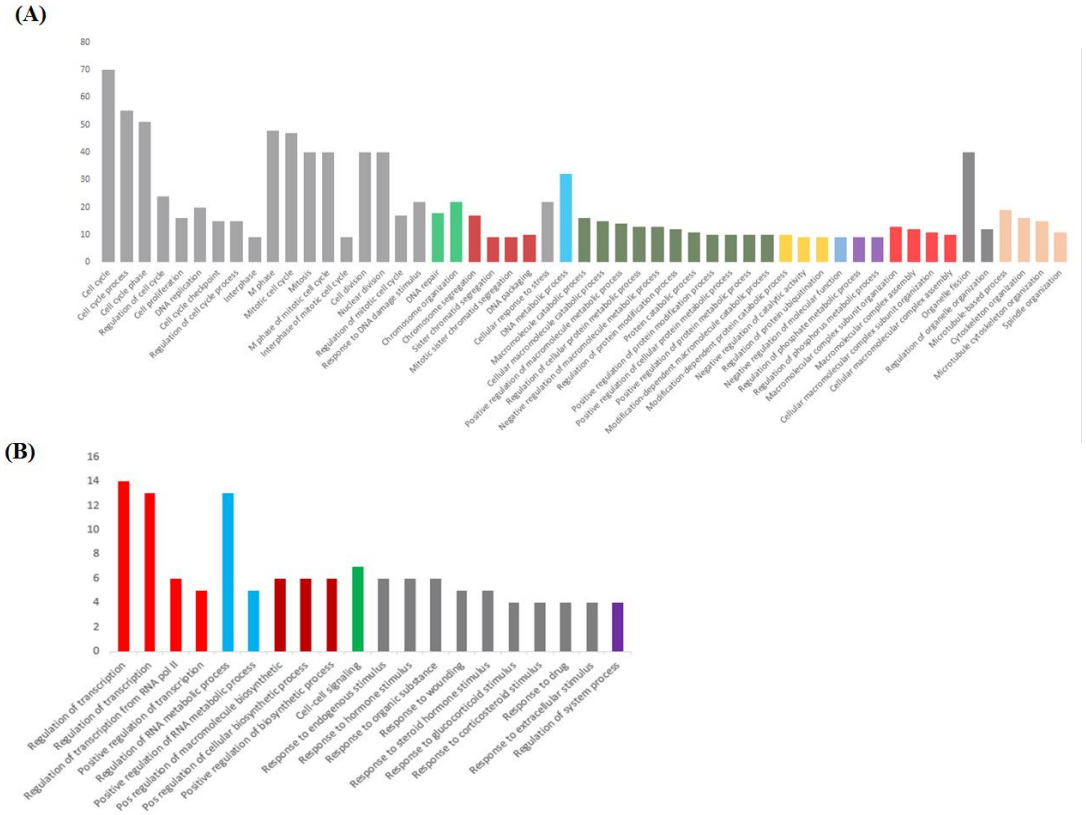


Figure 2 Gene ontology (GO) term enrichment analyses of the differentially expressed genes (DEGs). (A) Main upregulated GO terms and (B) GO terms of the downregulated DEGs.

Gene Enrichment and KEGG Pathway Analyses of DEGs

To investigate the roles and functions of deferentially expressed genes, the biological processes that Normal-CC DEGs participated in them were recognized by gene ontology analysis using DAVID (and BiNGO) functional annotation. In general, 160GO terms were identified, as detailed in Figure 2. Some of the important upregulated GO terms included the cell cycle pathway, cell division, DNA metabolic process, microtubule-based process, chromosome organization and response to DNA damage stimulus, among others. In addition, GO analysis demonstrated that the significantly enriched GO terms of downregulated DEGs included regulation of transcription, cell-cell signaling and regulation of RNA metabolic process. Moreover, 132 GO terms were obtained in the non-malignant-CC subjects. The significantly enriched GO terms of up/downregulated DEGs were mainly associated with similar to GO terms obtained from normal-CC patients.

To better understand the connections between DEGs and gene ontology terms, a DEG-GO network (Figure 3) was generated with the GO terms and DEGs using Cytoscape. Our results revealed that the crucial GO terms, determined as the extremely regulated by the DEGs, were involved in cell cycle (70 degrees).

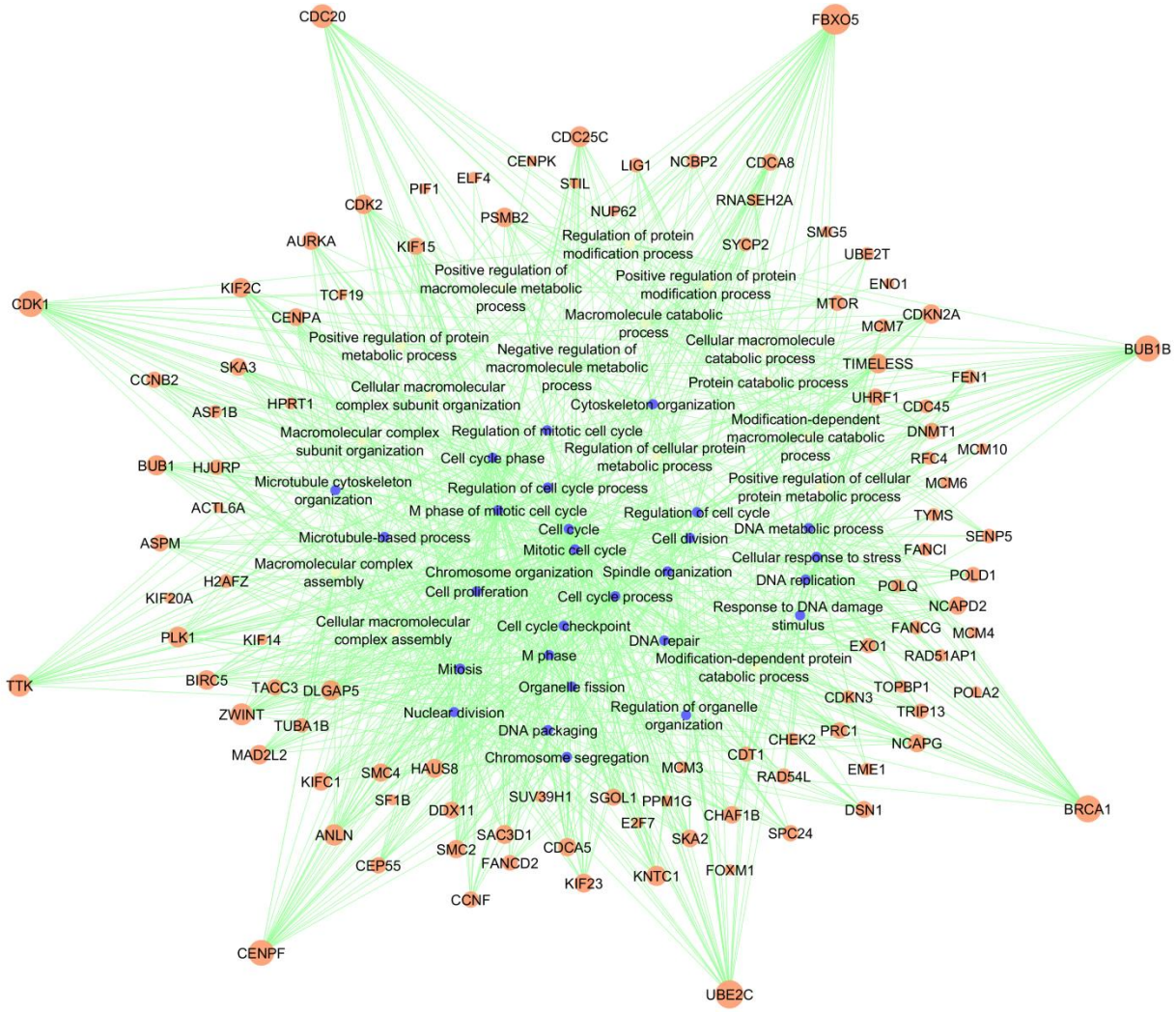
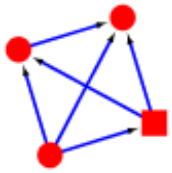
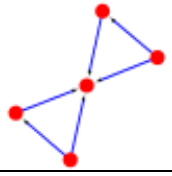
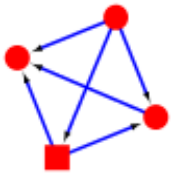
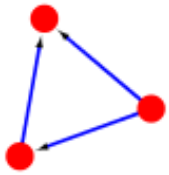


Figure 3 The differentially expressed genes-gene ontology-network (DEG-GO-Network). The orange squares in peripheral of network are DEGs, and the highlighted blue round spots are most important GOs (P value < 0.01 and FDR < 0.05). The lines represent the interactions between DEGs and GOs. The genes that regulate more GOs are FBXO5, UBE2C, BRCA1, CDK1, BUB1B, CENPF, CDC20, and TTK.

We identified molecular modules (clusters) that are highly connected within themselves. The results of MCODE for non-malignant tissue-CC network demonstrated that a gene module including of CDC45, MCM10, MCM3, MCM6, CDK2, and MCM7 has the highest calculated score among obtained modules (P-value<0.05 was considered significant). This gene cluster was also verified

by the result of cluster Maker analysis. Furthermore, the most important overrepresented module of normal-CC (MCM10, CDT1, ORC6, MCM4, CDK2, MCM3, MCM6, CDC45, MCM7) network recognized by both MCODE and cluster Maker plugins is functionally involved in the cell cycle process (Table 2). What is the biological role of these densely connected modules? We found that genes associated with each cluster have a consistent biological function and belong to the cell cycle functional class.

Table 2 Identified functional modules in non-malignant-CC and normal-CC networks (P-value <0.05)

Non-malignant-CC			
Module (Cluster)* No.	Score (Density of Nodes)	Nodes	Edge
(1) 	5.6	CDC45, MCM10, MCM3, MCM6, CDK2, MCM7	14
(2) 	4	NCAPG, SMC4, SMC2, NCAPD2	6
(3) 	3	CDK1, BUB1, CDC20, CCNE1, AR	6
Normal-CC			
(1) 	6.75	MCM10, CDT1, ORC6, MCM4, CDK2, MCM3, MCM6, CDC45, MCM7	27
(2) 	4	SMC2, NCAPD2, SMC4, NCAPG	6
(3) 	3	UHRF1, SUV39H1, DNMT1	3

* Nodes (genes) and edges (interactions) in identified clusters are shown in red and blue colors, respectively. CC: Cervical cancer

KEGG pathway analysis indicated that the significantly enriched pathways of upregulated DEGs were cell cycle, DNA replication, etc. (Table 3 and Figure 4). Hovewer, we found no important enriched pathways from downregulated DEGs.

Table 3 KEGG pathway analysis of upregulated differentially expressed genes (DEGs)

Pathway category and description	Count (%)	P-value
hsa04110: Cell cycle	17 (8.54)	4.31E-12
hsa03030:DNA replication	10 (5.0)	6.42E-10
hsa04114: Oocyte meiosis	12 (6.0)	1.87E-07
hsa04914: Progesterone-mediated oocyte maturation	7 (3.5)	0.001003415
hsa04115: p53 signaling pathway	6 (3.0)	0.002137648
hsa03430: Mismatch repair	4 (2.0)	0.003486834
hsa03440: Homologous recombination	3 (1.5)	0.054691405
hsa03410: Base excision repair	3 (1.5)	0.081075934

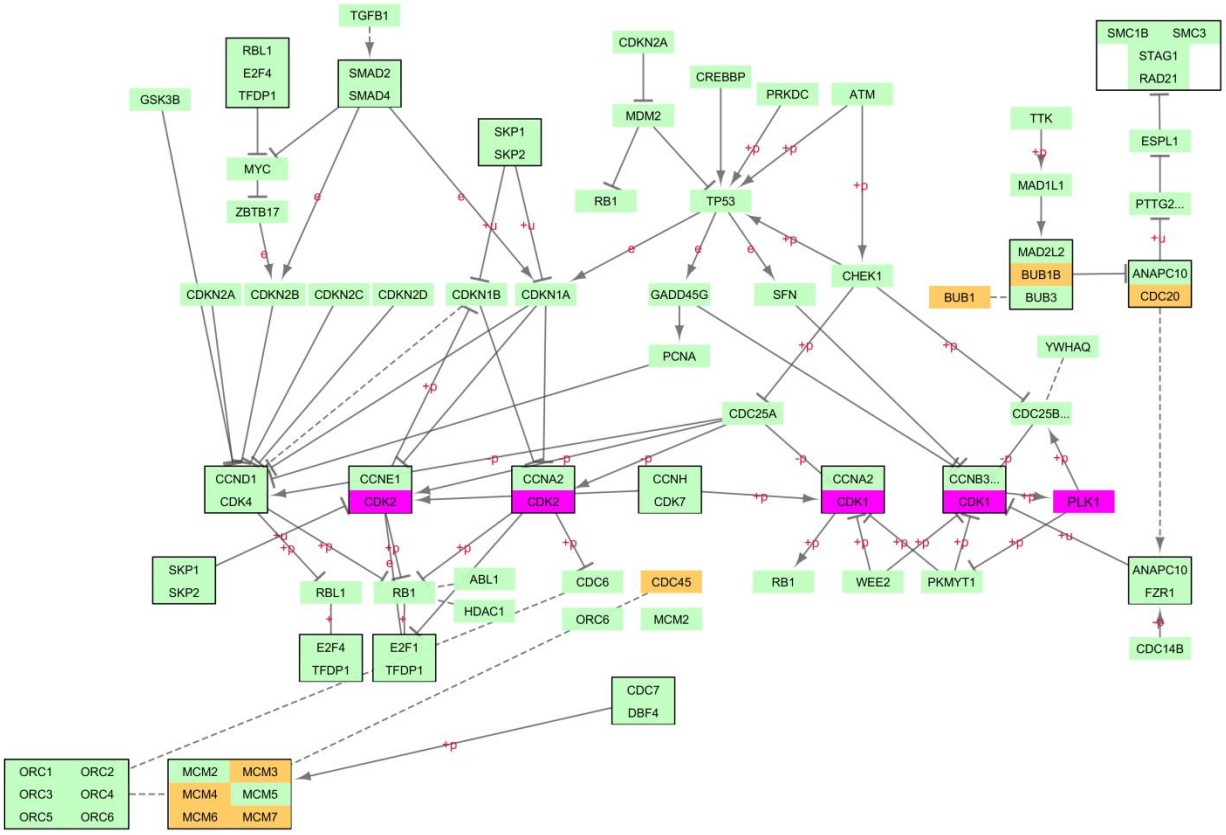


Figure 4 Cell cycle related genes which have been up-regulated in normal-CC network (orange). Most important hub genes (CDK1, CDK2 and PLK1) are shown in magenta color. The source of cell cycle pathway has been obtained from KEGG database. CC: Cervical cancer.

Detection of Essential Genes "hub genes" Involved in the Cervical Cancer

To predict the key regulators in the cervical cancer based on hub type variations, the stage-specific PPIs of cervical cancer were examined for critical genes, which had a high number of interactions. All generated PPIs were topologically evaluated using the Network Analyzer plugin of Cytoscape software. The Network Analyzer plugin calculates network centralities such as the distribution of node degrees (refers to the number of connections involving a node; it has been shown to correlate with the critical status of genes) (Jeong et al., 2001). Our result indicated that cyclin-dependent kinase 1 (CDK1) and CDK2 were determined as the most significant key genes of both non-malignant-CC- and normal-CC networks (Table 4). Also, our gene expression profile findings are consistent with this view in that other cell cycle associated genes including, *CDK2*, serine/threonine kinases Polo-like kinase 1 (PLK1), AURKA, BRCA1, and *CDC20* were all found highly critical genes in HPV16-infected CC. Among highest rank of genes from normal-non-malignant PPI, 73.3% of the genes exhibit similar patterns in normal-CC PPI.

Table 4 The Top 20 Hub Genes Obtained from analysis of the normal-non-malignant and non-malignant-CC networks

Non-malignant-CC				Normal-CC			
Gene Name	Degree	Betweenness Centrality	Closeness Centrality	Gene Name	Degree	Betweenness Centrality	Closeness Centrality
CDK1	25	0.307	0.47	CDK1	27	0.32	0.48
CDK2	25	0.27	0.47	CDK2	25	0.18	0.44
PLK1	17	0.187	0.42	PLK1	21	0.26	0.45
AURKA	14	0.107	0.37	BRCA1	18	0.26	0.44
RPA3	13	0.13	0.34	CDC20	13	0.087	0.42
MCM6	12	0.07	0.39	MCM6	13	0.036	0.36
CDC20	10	0.087	0.42	MCM7	11	0.018	0.38
MCM7	9	0.018	0.39	AURKA	11	0.07	0.38
MCM3	9	0.05	0.41	MCM4	10	0.011	0.37
CDC45	9	0.04	0.37	CDC45	10	0.014	0.35
GMNN	7	0.016	0.38	MCM3	9	0.024	0.38
CCNE1	7	0.011	0.39	BUB1	9	0.11	0.39
BUB1	7	0.05	0.37	BUB1B	9	0.035	0.36
ECT2	7	0.16	0.43	CDT1	8	0.0039	0.37
CDKN2A	6	0.033	0.33	ECT2	8	0.12	0.40

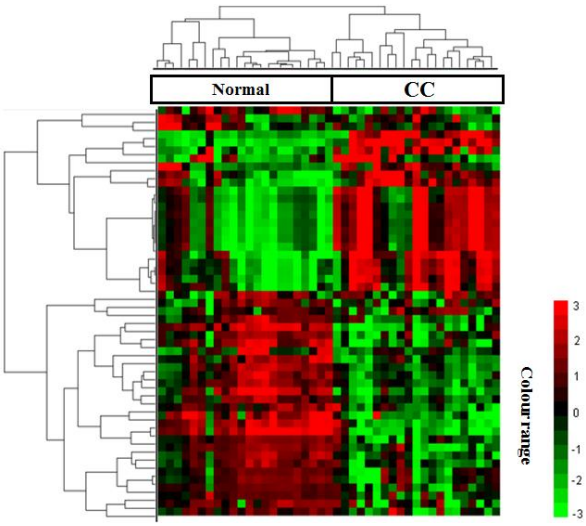


Figure 5 Hierarchical clustering and TreeView analysis of DEGs between normal (n = 24) and CC (n = 19) samples obtained from independent dataset. Fifthy-one differentially expressed genes at P≤0.01 and fold change 3 was used for the analysis. The color indicates the level of mRNA expression is as follow: red - higher level of expression (upregulation), green - lower level of expression (downregulation), black - no expression changes. The clustergram separates the samples from women with neohistologicaly normal and CC. CC: Cervical cancer.

Verification of the Representative PPI

For verification of the strengths of gene expression profiles of the PPI network and hub variation, we examined another independent microarray gene expression dataset including pathological stage annotation to the expand of oroginal dataset. We performed microarray data from GSE9750 to confirm our normal-CC network. To verify the expression profiles of the normal-CC network, we analyzed the genes to GSE9750 dataset. The majority of the normal-CC network genes revealed undeviating expression changes in this independent dataset. Additionally, gene enrichment was performed for normal-CC network. The result indicated that majority of nodes was significantly enriched in cell cycle patway and regulation. Furthermore, we revealed that most of the original normal-CC PPI genes are present in the validation network (Figure 5). Moreover, most important hub genes detected in original network are hubs in the verification network, comprising of CDK1, CDC20, MCM6, AURKA and MMP7. Interestingly, CDK1 identified as a critical hub gene in both original and verification networks.

Detection of HPV interacting proteins

Hu-Vir pairwise protein-protein interaction (PPI) were combined and created from PIG and Virus MINT datasets (Figure 6). It is interesting to note that CDK1 and CDK2, hub genes, are targeted. HPV protein E7 appear to be importantly involved in these interactions. E6 and E7 proteins acts mainly as an oncoprotein by stimulating the suppression of many host cell key regulatory proteins. In addition, Hu-Vir PPI network analysisrevealed that HPV protein E6 and E7 can interact with breast cancer type 1 susceptibility protein (BRCA1). BRCA1 act as an E3 ubiquitin-protein ligase activity, which is required for its tumor suppressor function. A formentaioed viral proteins could deregulate the crucial cellular functions such as cell cycle pathway and cell division by interacting with the hub proteins in the protein-protein interaction network.

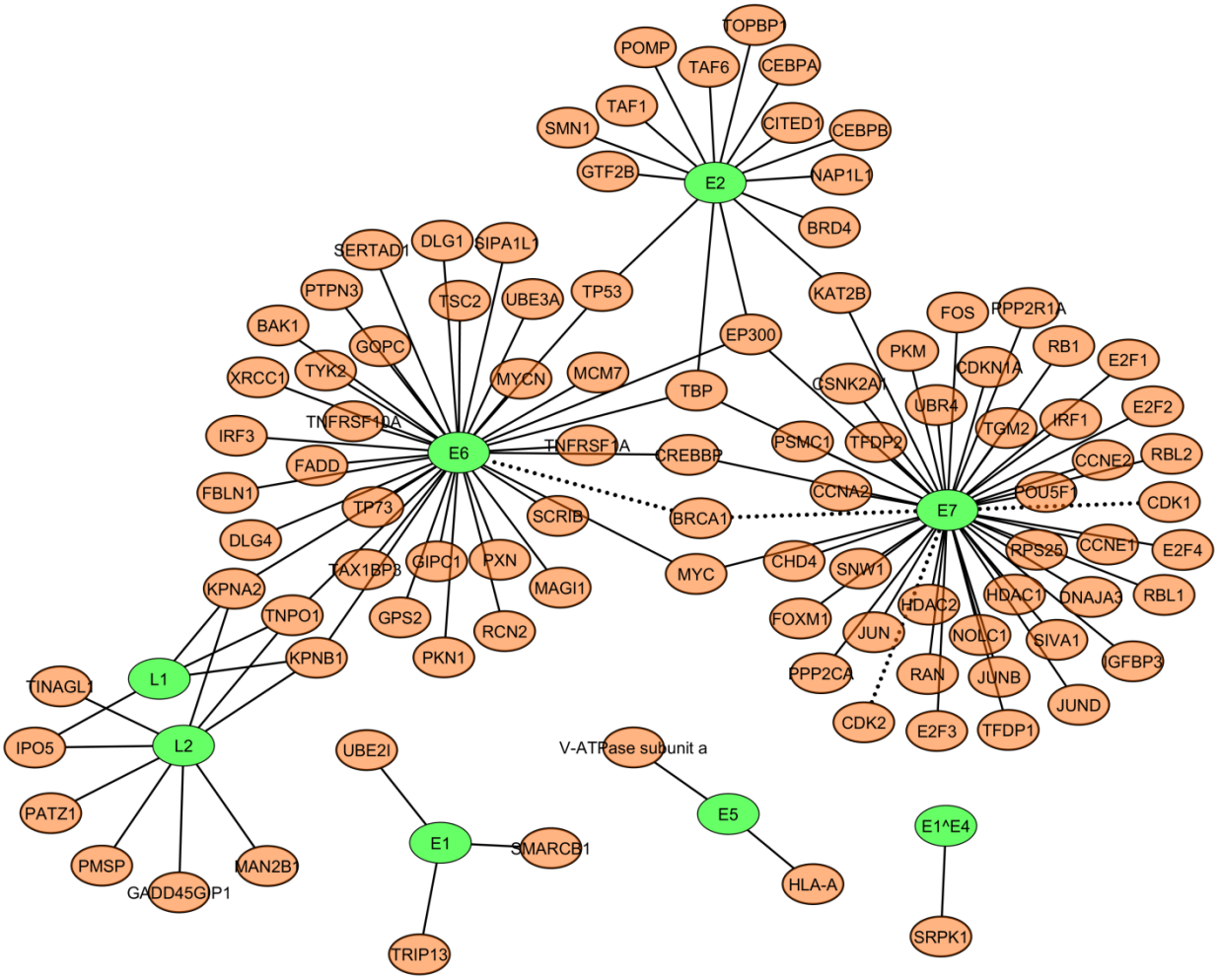


Figure 6 Human-HPV 16 network generated from Hu-Vir database. HPV proteins are shown in green color. The human protein targets existed in most important hub genes of network are indicated in doted lines.

4. DISCUSSION

Gene Expression Omnibus is a source of expression profiles and large public repositories of gene expression profiles. High throughput transcriptome techniques allow standard quantity of expression profiles and functional interactions from the cells and tissues of many different organisms. The present study provides the network-based analysis from KEGG molecular pathways or gene ontology (Kanehisa & Goto, 2000; Ashburner et al., 2000) in healthy women and HPV16 positive cervical cancer subjects. Moreover, the microarray data were integrated with high throughput PPI data that were investigated for deregulated networks among normal-non-malignant HPV positive and CC patients. Our PPI data facilitated the representation of overriding finding. One important aspect of the HPV 16 gene expression pattern was the significant upregulation of genes involved in the cell cycle process.

Present PPI network indicated that upregulation of the cell cycle related-genes occured in the tissue samples of cervical cancer. Remarkably, functional modules and GO analyses of cervical cancer was considerably associated with cell cycle. Molecular network results also showed that CDK1, CDK2, and PLK1 were the most important "hub" genes participated in cell cycle progression in the cervical cancer. Surprisingly, analysis of another independent microarray gene expression dataset demonstrated that CDK1 is most important hub gene in verification network. To the best of our knowledge, this is the first evidence illustrating that these genes are highlighted in a systematic manner in HPV16 studies. CDKs are the positive key regulators that orchestrate activities throughout the cell cycle process (Figure 7). CDKs carry out this function by phosphorylating a diverse array of proteins. Each phosphorylation event occurs at an appropriate point during the cell cycle, there by stimulating or inhibiting a particular cellular process involved in cell division (Karp, 2013). The CDK1, formerly called CDC2 (or p^{34Cdc2}), is the major facilitator for G1 progress and G1-S transition via association with multiple interphase cyclins (Enserink & Kolodner, 2010). Surprisingly, several evidences indicate that CDK1 is the only essential member of the CDK subfamily for cell division and progression (Malumbres & Barbacid, 2009). Deletion of the CDK1 gene cannot be rescued by knocking-in of its relative G1 CDKs, and mouse knockouts of CDK2, CDK3, CDK4, or CDK6 are viable (Berthet et al., 2003; Ye et al., 2001; Tsutsui et al., 1999; Hu et al., 2009). Furthermore, CDK2 is activated by interaction with cyclin E and cyclin A during cell cycle, which permits the G1-S transition to promote the E2F transcriptional pathway, early stages of DNA synthesis, and modulating G2 phase progression (Hochegger et al., 2008). Previous *in vitro* study indicated that both CDK1 and CDK2 are increased in E7-expressing cells (spontaneously immortalized human keratinocyte cell line, NIKS) upon DNA damage. Fan et al., demonstrated that CDK1 plays an important role in abrogating and bypassing the G1 check point in E7-expressing NIKS cells (Fan & Chen, 2014). Based on our data, CDK1 and CDK2 are evidently important hub gene in the cervical cancer invasion, which may provide development of novel therapeutics. Accordingly, CDK inhibition has been suggesteed as a potential therapeutic target for cancer treatment (Stone et al., 2012). Our results also indicated that mitotic cyclin-independent serine/threonine kinases Polo-like kinase 1 (PLK1) is closely associated with development and progression of cervical cancer. The PLK1, which is a potential target for cancer therapy, is a key regulator of cell division in eukaryotic cells (Cheng et al., 2015). Zhang et al., revealed that PLK1 knockdown induces accumulation of HeLa cells in the G2/M phase of the cell cycle and enhances cisplatin-induced apoptosis (Zhang et al., 2009).

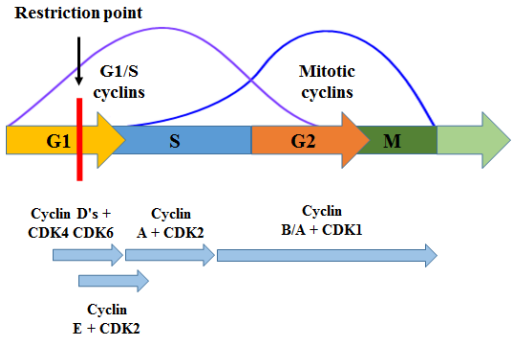


Figure 7 Combinations between various cyclins and CDKs at different phases in the mammalian cell cycle.

In our study, we observed a significantly upregulated mRNA level of BRCA1 in CC tissues compared to normal cervix. Previous studies on BRCA1 have indicated that this protein is involved in DNA double-strand break repair, cell cycle arrest and apoptosis (Roy et al., 2012). However, Kaczkowski et al., reported a group of genes such as BRCA1 downregulated in HPV16 transfected human keratinocyte cell line (HaCaT) (Kaczkowski et al., 2012). On the other hand, Thomas et al., indicated that BRCA1 expression was 9.2-fold increased in cervical cancer in copmarsion with histologically normal tissue (Thomas et al., 2013). Upregulated expression level

of BRCA1 has been reported in sporadicepithelial ovarian cancer and lung cancer, which isas an indicator of chemoresistance (Taron et al., 2004; Clark-Knowles et al., 2010). Interetstingly, Balacescu et al., revealed a significantly increased expression level of BRCA1in the non-complete treatment response compared to complete response cervical tumor tissue (Balacescu et al., 2014). Previous BRCA pathway studies and molecular profiling have demonstrated that BRCA-deficient cells are incapable of repairing DNA double strand break by homologous recombination, therefore, more sensitive to chemotherapeutic agents (Roy et al., 2012; Rigakos & Razis, 2012).

Interestingly, previous study demonstrated that CDK, seemed to play an important role inthe cell cycle regulation of Epstein-Barr Virus (EBV) positive-infectious mononucleosis disease (Poorebrahim et al., 2017). Like HPV, other viruses including, hepatitis B virus (Bouchard et al., 2001), hepatitis C virus (Munakata et al., 2005) and human T-cell leukemia virus type 1 (HTLV-1) (Wang et al., 2002) might have evolved various strategies to deregulate the cell cycle. In our study, however, more information and experimental validation required for our claims. Nonetheless, our network-based study renders a model to extract information from high appropriate genomic microarray in human specimens.

In line with this result, DAVID functional groups and pathways enriched by DEGs in cervical cancer indicated that cell cycle and proliferation were upregulated (Figure 3). Likewise, pathways such as response to DNA damage stimulus, DNA metabolic process, and mitotic cell cycle were associated with highly proliferative cells. Interestingly, functional modules and their related gene analysis of cervical cancer disease indicated that these genes were predominantly involved in the cell cycle pathway (Table 3).

A previous study has shown that the HPV proteins disrupt cell cycle phases by interaction with cellular proteins. Our findings recommend that deregulations of these cellular processes may be the result of direct HPV protein interactions with the DEGs in the molecular networks (Figure 7). The most important viral proteins in those interactions are E6, E7, E1, E2, L1 and L2. Stabilization of specific viral mRNAs appears to be critical in the development of cervical carcinoma associated with infection by high-risk human papilloma viruses. The E6 and E7 proteins of HPV16 induce abnormal cell proliferation. E6 and E7 oncogenes make these functions available by localizing to the nucleus and binding to cell cycle regulatory proteins. E6 binds to p53, a tumor suppressor protein, inducing its degradation, and E7 binds to pRb–E2F,a tumor suppressor complex, leading to their dissociation. The consensus of these interactions is the transition of the infected cell to the S phase of cell cycle (Yim & Park, 2005; Munger & Jones, 2015). Our Hu-Vir network revealed direct interaction of HPV16 proteins with CDK1, CDK2, and BRCA1 hub proteins, which could deregulate cell cycle pathway. HPV protein E7 directly interacts with CDK1 and CDK2 proteins. Zhang et al., also reported that the E6 and E7 HPV oncoproteins interact with BRCA1 and alter its activity in cervical cancer cells (Zhang et al., 2005). It seems that a hub targeting mechanism may show a more effective approach for viruses to overcome host's cellular machineries.

5. CONCLUSION

In the present study, important differentially expressed genes were detected by comparing gene expression profiles between histologically normal and cervical cancer tissues. The most important finding of our study was that CDK1, CDK2, and PLK1 play critical roles in modulating integrative genetic networks involved in the development and invasion of cervical cancer. These hub genes might help improve pathogenesis of CC and may serve as a diagnosis marker for the treatment of cervical cancer.

Conflict of interest

None of the authors declared any conflict of interest.

Acknowledgements

This study has been funded and supported by Tehran University of Medical Sciences (TUMS), Grant no. 39293. It has also been part of a Ph.D. thesis supported by Tehran University of Medical Sciences.

List of abbreviations

CDK: Cyclin-dependent kinase; PLK1: polo-like kinase 1; FBXO5: F-box only protein 5; UBE2C: Ubiquitin-conjugating enzyme E2 C; BRCA1: Breast cancer type 1 susceptibility protein; BUB1B: BUB1 Mitotic Checkpoint Serine/Threonine Kinase B; CENPF: Centromere Protein F; CDC20: Cell-division cycle protein 20; TTK: TTK protein kinase; MCM: Mini-chromosome maintenance proteins; CDT1: Chromatin licensing and DNA replication factor 1; ORC6: Origin recognition complex subunit 6; NCAPG: Non-SMC condensin I complex subunit G; NCAPD2: Non-SMC condensin I complex subunit D2; CCNE1: Cyclin E1; AR: Androgen receptor; UHRF1: Ubiquitin-like, containing PHD and RING finger domains, 1; SUV39H1: Suppressor of Variegation 3–9 Homolog 1; DNMT1: DNA

(cytosine-5)-methyltransferase 1; AURKA: Aurora kinase A; GMNN: Geminin, DNA replication inhibitor; ECT2: Epithelial cell transforming protein 2; CDKN2A: cyclin-dependent kinase Inhibitor 2A; MMP7: Matrix metalloproteinase-7.

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